REMARKS

Conventional methods for measuring malaria parasites by flow cytometry—such as the methods described in *Saito-Ito et al.* (*Parasitology International* **2001**, *50*, 249-257) and in *van Vianen et al.* (*Cytometry* **1993**, *14*, 276-280)—involve hemolysis of erythrocytes using only a single surfactant to release malaria parasite. As described in the specification (e.g., page 5, lines 11-17; page 10, lines 2-9; page 32, line 10 to page 34, line 22), these methods suffer from a serious drawback in that reticulocytes that have not undergone sufficient hemolysis appear in a region of a scattergram that overlaps with a region in which malaria parasites to be detected are located.

In contrast to the above-described conventional methods, the claimed invention provides methods wherein a specimen is treated with a first hemolytic agent comprising a first surfactant and a second hemolytic agent comprising a second surfactant having a hemolytic activity higher than that of the first surfactant. By conducting the hemolysis of an erythrocyte in two steps, the effect of reticulocyte may be eliminated to enable more accurate detection of malaria parasite (e.g., specification, page 10, lines 9-14).

As further explained below, neither *Saito-Ito et al.* nor *van Vianen et al.*, alone or in combination, teaches or suggests the use of a first hemolytic agent comprising a first surfactant and a second hemolytic agent comprising a second surfactant, as required by the claimed invention. Moreover, neither *Saito-Ito et al.* nor *van Vianen et al.*, alone or in combination, teaches or suggests a second surfactant having a hemolytic activity higher than that of the first surfactant, as further required by the claimed invention.

Election/Restriction

In confirmation of their telephonic election, Applicants hereby elect without traverse to prosecute the invention of Group I (claims 1-9) drawn to methods for detecting malaria parasites.

Non-elected claims 10-22 drawn to the inventions of Groups II and III have been canceled by the present amendment without prejudice to their continued prosecution in a continuation and/or divisional application.

Claim Rejections – 35 U.S.C. § 112, Second Paragraph

The rejection of claims 5-9 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention is respectfully traversed.

Applicants respectfully submit that the meaning of the phrase "wherein a zone" recited in dependent claim 8 would have been abundantly clear to one of ordinary skill in the art in view of the description provided in the specification (e.g., page 24, line 23 to page 25, line 8; page 31, line 21 to page 32, line 8).

Likewise, Applicants respectfully submit that the meaning of the phrases "within a range" and "within the range" recited in dependent claim 9 would have been abundantly clear to one of ordinary skill in the art in view of the description provided in the specification (e.g., page 26, lines 11-25; page 37, line 2 to page 38, line 24).

Applicants note that none of dependent claims 5, 6, and 7 recites any of the above-identified phrases. Accordingly, Applicants believe that the rejection of these claims under 35 U.S.C. § 112, second paragraph was made in error.

For at least the reasons set forth above, Applicants submit that the claims are definite and particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Claim Rejections – 35 U.S.C. §§ 102(b) and 103(a)

1. *Saito-Ito et al.*

The rejection of claims 1-9 under 35 U.S.C. § 102(b) as being anticipated by *Saito-Ito et al.* is respectfully traversed.

Each of independent claims 1 and 5 recites treating a specimen or sample with a first hemolytic agent comprising a first surfactant and a second hemolytic agent comprising a second surfactant. In addition, each of independent claims 1 and 5 recites that the second surfactant has a hemolytic activity higher than that of the first surfactant. In contrast, *Saito-Ito et al.* describes the use of only a single surfactant and does not teach or suggest a second hemolytic agent comprising a second surfactant, as required by the claimed invention. Moreover, *Saito-Ito et al.* likewise does not teach or suggest a second surfactant having a hemolytic activity higher than that of the first surfactant, as further required by the claimed invention.

Saito-Ito et al. describes a flow cytometric system for the detection of *Plasmodium falciparum* that involves lysing erythrocytes and staining parasites simultaneously using a hemolysing and staining solution containing dodecyl methyl ammonium chloride and acridine orange (e.g., page 249, Abstract, lines 1-3; page 250, col. 1, last two sentences of Introduction). The methods reported in *Saito-Ito et al.* are limited to hemolyses using only a single surfactant (i.e., dodecyl methyl ammonium chloride)—not two surfactants as required by the claimed invention. [It is noted that the EDTA added to blood samples in *Saito-Ito et al.* is an anticoagulant—not a surfactant as suggested by the Examiner (e.g., Office Action, page 6). Indeed, as noted in the specification (e.g., page 10, line 25 to page 11, line 4), EDTA may also be used as an anticoagulant in accordance with the claimed invention.] In addition to being silent with respect to the use of a second surfactant, *Saito-Ito et al.* is likewise silent with respect to the use of surfactants having different hemolytic activities, and does not teach or suggest a second surfactant having a hemolytic activity higher than that of a first surfactant, as further required by the claimed invention.

As noted in *Saito-Ito et al.* (e.g., page 257, second sentence under Acknowledgements), the single-surfactant methods described therein are the subject of Japanese published unexamined patent application No. 11-75892. The single-surfactant methods of *Saito-Ito et al.* and of Japanese application No. 11-75892 are described in the specification and contrasted with the claimed invention (e.g., page 6, lines 7-20; page 10, lines 2-14; Comparative Examples 1-3, page 32, line 10 to page 40, line 6). For example, as described in the specification (e.g., page 10, lines 2-14, emphases added):

> It was found that that when the methods described by A. Saito-Ito (*Parasitol. Int.*) and in Japanese Unexamined Patent Publication No. 11-75892 were applied to the measurement of blood from an actual malaria-infected patient, reticulocyte still exists that has not undergone sufficient hemolysis, thereby resulting in increased background which makes it difficult to conduct correct measurements of the specimen. However, in accordance

> with the present invention, it was discovered that by conducting the hemolysis of an erythrocyte in two steps, the effect of reticulocyte can be fully eliminated, thereby enabling correct and highly accurate detection of the malaria parasite.

Moreover, as described in Comparative Example 1 of the specification, when only a single surfactant was used to treat a specimen as per the methods of *Saito-Ito et al.*, a large number of reticulocytes did not undergo sufficient hemolysis; these reticulocytes are clearly shown in the dotted-line oval region of FIG. 2 (e.g., page 33, lines 3-5). As a result, false detection of malaria parasites in non-malaria specimens and reductions in measurement sensitivity may result (e.g., specification, page 5, lines 11-17; page 33, lines 6-10). Moreover, attempts to correct this problem by increasing surfactant concentration in an effort to promote reticulocyte hemolysis failed because the increased surfactant concentrations also promoted dissolution of malaria parasite (e.g., specification, page 33, line 18 to page 34, line 22).

The above-described problems associated with single-surfactant hemolysis were recognized and acknowledged in *Saito-Ito et al.*, which states, for example, that "the method could not be applied to the analysis of *Plasmodium*-infected mice ... [b]ecause particles that seemed to be residual bodies of nuclei remained and were plotted in the parasite area of the scattergram when erythrocytes of mice were treated by the hemolysing and staining solution (e.g., page 256, last two sentences of column 1).

As clearly demonstrated by Example 1 and Comparative Examples 1-3 of the specification (page 30, line 20 to page 40, line 6), the methods of the claimed invention whereby a specimen is treated with a first hemolytic agent comprising a first surfactant and a second hemolytic agent comprising a second surfactant having a hemolytic activity higher than that of the first surfactant enable sufficient hemolysis of erythrocytes and reticulocytes in a sample without causing damage to malaria parasites that would affect measurement (e.g., specification, page 40, lines 1-6).

In contrast to the claimed invention, *Saito-Ito et al.* does not teach or suggest a first hemolytic agent comprising a first surfactant and a second hemolytic agent comprising a second surfactant. Moreover, *Saito-Ito et al.* likewise does not teach or suggest a second surfactant having a hemolytic activity higher than that of the first surfactant. For at least these reasons, Applicants respectfully submit that the claimed invention is neither anticipated by nor would have been obvious in view of *Saito-Ito et al.* Accordingly, withdrawal of this ground of rejection is respectfully requested.

2. *van Vianen et al.*

The rejection of claims 1-9 under 35 U.S.C. § 102(b) as being anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as being obvious over *van Vianen et al.* is respectfully traversed.

As noted above, each of independent claims 1 and 5 recites treating a specimen or sample with a first hemolytic agent comprising a first surfactant and a second hemolytic agent comprising a second surfactant. In addition, each of independent claims 1 and 5 recites that the second surfactant has a hemolytic activity higher than that of the first surfactant. In contrast, *van Vianen et al.* describes the use of only a single surfactant and does not teach or suggest a second hemolytic agent comprising a second surfactant, as required by the claimed invention. Moreover, *van Vianen et al.* likewise does not teach or suggest a second surfactant having a hemolytic activity higher than that of the first surfactant, as further required by the claimed invention.

van Vianen et al. describes a flow cytometric screening method for detecting malaria parasites that involves treating blood samples with a FACS lysing solution (e.g., page 276, Abstract, lines 4-9; page 277, col. 1, first paragraph under Materials and Methods). The methods reported in *van Vianen et al.* are limited to hemolyses using only a single surfactant (i.e., FACS lysing solution)—not two surfactants as required by the claimed invention. In addition to being silent with respect to the use of a second surfactant, *van Vianen et al.* is likewise silent with respect to the use of surfactants having different hemolytic activities, and does not teach or suggest a second surfactant having a hemolytic activity higher than that of a first surfactant, as further required by the claimed invention.

As described in the specification (e.g., page 10, lines 9-14; page 30, line 20 to page 40, line 6) and as noted above in the arguments addressing the *Saito-Ito et al.* reference, the use of a first hemolytic agent comprising a first surfactant and a second hemolytic agent comprising a second surfactant, wherein the second surfactant has a hemolytic activity higher than that of the first surfactant, provides significant advantages over conventional methods whereby samples are treated with only a single surfactant. By way of example, the undesirable overlapping of reticulocyte and malaria parasite in scattergrams may be minimized, thereby enabling more accurate and sensitive detection of malaria parasites.

Inasmuch as *van Vianen et al.* neither teaches or suggests a first hemolytic agent comprising a first surfactant and a second hemolytic agent comprising a second surfactant, and neither teaches nor suggests a second surfactant having a hemolytic activity higher than that of the first surfactant, as required by the claimed invention, Applicants respectfully submit that the claimed invention is neither anticipated by nor would have been obvious in view of *van Vianen et al.* Accordingly, withdrawal of this ground of rejection is respectfully requested.

New Claims

New claims 23-35 are dependent claims that depend from one or both of independent claims 1 and 5. Inasmuch as independent claims 1 and 5 are neither anticipated by nor would have been obvious in view of *Saito-Ito et al.* and *van Vianen et al.*, alone or in combination, Applicants respectfully submit that new dependent claims 23-35 are in condition for allowance as written.

Conclusion

In view of the Remarks set forth above, Applicants respectfully submit that the claimed invention is in condition for allowance. Early notification to such effect is earnestly solicited.

If for any reason the Examiner feels that the above Remarks do not put the claims in condition to be allowed, and that a discussion would be helpful, it is respectfully requested that the Examiner contact the undersigned agent directly at (312)-321-4257.

Respectfully submitted,

Gregory Zayia

Gregory H. Zayia
Registration No. 48,059
Agent for Applicants

BRINKS HOFER GILSON & LIONE
P.O. BOX 10395
CHICAGO, ILLINOIS 60610
(312) 321-4200